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Research report

Brain enlargement and increased behavioral and cytokine reactivity in infant monkeys following acute prenatal endotoxemia

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ABSTRACT

Infections and inflammatory conditions during pregnancy can dysregulate neural development and increase the risk for developing autism and schizophrenia. The following research utilized a nonhuman primate model to investigate the potential impact of a mild endotoxemia during pregnancy on brain maturation and behavioral reactivity as well as the infants' hormone and immune physiology. Nine pregnant female rhesus monkeys (*Macaca mulatta*) were administered nanogram concentrations of lipopolysaccharide (LPS) on two consecutive days, 6 weeks before term, and their offspring were compared to nine control animals. When tested under arousing challenge conditions, infants from the LPS pregnancies were more behaviorally disturbed, including a failure to show a normal attenuation of startle responses on tests of prepulse inhibition. Examination of their brains at 1 year of age with magnetic resonance imaging (MRI) revealed the unexpected finding of a significant 8.8% increase in global white matter volume distributed across many cortical regions compared to controls. More selective changes in regional gray matter volume and cortical thickness were noted in parietal, medial temporal, and frontal areas. While inhibited neural growth has been described previously after prenatal infection and LPS administration at higher doses in rodents, this low dose endotoxemia in the monkey is the first paradigm to produce a neural phenotype associated with augmented gray and white matter growth.

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1. Introduction

Prenatal infections and inflammatory responses during pregnancy can result in adverse effects on brain development that contribute to the etiology of affective disorders or neurodevelopmental pathologies like autism and schizophrenia [1–3]. One behavioral feature common to these disorders is a heightened reactivity to visually or acoustically arousing stimuli, often manifest by greater anxiousness and impaired sensory-motor integration, which is evident on prepulse inhibition (PPI) tests of attention and startle [3–5]. In addition, many prenatal challenge studies in rodents and nonhuman primates have found that the offspring exhibit abnormal neuroendocrine and immune responses [6–8].

Developmental abnormalities caused by prenatal infections can be mimicked by the administration of noninfectious agents that evoke inflammatory responses, such as polyriboinosinic-polyribocytidilic acid (poly I:C) or lipopolysaccharide (LPS) [9–12]. Therefore, it appears that one common mediating pathway initiating this pathogenesis is a disruption of placental functioning by proinflammatory cytokines [13,14]. In turn, cascading effects on the developing brain impact postnatal behavior in ways that resemble dysfunctional features seen in autism and schizophrenia [2,11,15]. Teratogenic disruptions of synapse formation, cellular proliferation, and myelination may promote these abnormal neural phenotypes [16,17]. For example, prenatal LPS treatment in rodents induces white matter (WM) abnormalities and reduced myelin density in the corpus callosum, as well as in cortical and subcortical regions [15–19]. Poly I:C administration also reduces gray matter (GM) in medial temporal areas such as entorhinal cortex and hippocampus [9,20]. In addition, influenza virus infections in pregnant mice result in pups with reduced cortical thickness in the frontal lobe [6], and juvenile rhesus monkeys exposed to influenza prenatally have smaller GM volumes in several cortical regions, including frontal, temporal and parietal cortices [21]. Yet, while prenatal infection typically inhibits neural growth and prolif-

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eration, increased GM and WM in certain regions have been found in many individuals with autism – at least early in development [22,23].

To replicate and extend behavioral and neural findings from rodents and nonhuman primates, we sought to model a moderate, self-limiting bacterial infection during pregnancy by administering LPS to gravid female monkeys. Because monkeys and humans are comparatively sensitive to LPS, a low dose protocol was employed to minimize the overt pathology that occurs when high concentrations damage the placenta and fetal brain [24]. Prior research in juvenile monkeys has shown that 4 ng/kg of LPS induces transient increases in IL-6 and cortisol for up to 24 h [25]. The offspring from the prenatal LPS condition were then assessed longitudinally across the first 1.5 years of life. The *a priori* prediction was that infants from an endotoxin-challenged pregnancy would: (1) be more behaviorally reactive; (2) manifest signs of neuroendocrine and immune dysregulation; (3) show changes in cortical volume and thickness in sensitive brain areas related to emotional regulation and attention. Although we found the expected effects on behavior, the brains of monkeys from LPS pregnancies showed robust increases in WM volumes and selective GM changes in parietal and temporal regions [22]. This novel brain phenotype may be useful for investigating certain neurodevelopmental disorders like autism.

2. Methods

2.1. Animals and prenatal treatments

Details of the LPS administration to gravid females and effects on leukocyte demargination and circulating IL-6 levels are provided in Supplement Table 1. Briefly, nine pregnant monkeys (*Macaca mulatta*) received two intravenous injections of LPS each morning on Days 125 and 126 of their 169-day pregnancy (2 ng/kg, $n = 1$; 4 ng/kg, $n = 8$). Viral infection during this prenatal period had been found to induce neurodevelopmental and behavioral alterations [21]. The lower 2 ng/kg dose was used just once to assess toxicity and possible risk of miscarriage. Two other gravid females were piloted at the 4 ng/kg dose before the remaining 6 females in this condition were administered with LPS. The nine control mothers were either administered identical volumes of saline ($n = 2$) or not handled ($n = 7$), neither of which significantly affected maternal IL-6 or leukocyte counts. Gestational length, neonatal weight and subsequent growth after birth were determined. Both prenatal conditions included similar numbers of male and female offspring ($n = 8$ and 10, respectively). The schedule of postnatal testing is summarized in Supplement Table 2. After it was determined that growth patterns and health among 3 initial LPS offspring were not greatly perturbed, additional testing incorporating PPI, cortisol assessment, and *in vitro* cellular stimulation assays was completed on the remaining cohort of subjects (LPS = 6, Control = 9). Researchers who collected behavioral and MRI data, as well as assayed physiological samples, were blind to the monkeys' prenatal conditions. All experimental procedures were approved by the Institutional Animal Care and Use Committee and the Office of Biological Safety at the University of Wisconsin—Madison.

2.2. Behavioral assessments

2.2.1. Neonatal, mother-Infant, and peer behavior

Temperament, neuromotor reflexes, and attentional responses were assessed at 2 weeks of age using the Infant Behavioral Assessment Scale (IBAS), adapted from Brazelton [26] for use in monkeys [27,28]. Factor loadings of 4 IBAS categories, which are based on 29 test items, are described in detail in a recent report from our group [29]. Social interactions were observed between each infant and its mother from 1 to 4 months and with peers from 6 to 7 months during 12 non-contiguous 5-min periods per month.

2.2.2. Human Intruder Paradigm

Stress reactivity was assessed at 8–9 months of age using a modified version of the Human Intruder Paradigm [25,30]. Briefly, hostile and fearful behaviors defined in Supplement Table 3 were assessed during 5 stare (SC) and no eye contact (NEC) trials, each of 5 min duration.

2.2.3. Prepulse inhibition

Using a PPI protocol for monkeys [31], responses to acoustical startle and the extent of adaptation after pairing the stimulus probe with a softer prepulse sound were assessed at 10–12 months of age. Each infant was tested in a sound-attenuating booth while freely moving in a small cage. Shifts in movement were recorded along 3 dimensions and computed as $x^2 + y^2 + z^2$ to remove direction effects. Startle sounds were played at either 105 or 115 dB for 40 ms. The prepulse was an 80 dB broadcast for 20 ms at intervals of 45, 120, or 500 ms before the startle probe. After a 15 min acclimation, each set of prepulse and startle probes was repeated four times across the 1 h test. Acceleration was used to index startle to remove the influence of body weight. Percent reduction in startle after prepulses, as compared to the trials with the startle probe alone, was computed using the following formula: (100 × [(mean Probe alone trials − mean Prepulse and Probe trials)/mean Probe alone trials]).

2.3. Physiological assessments

2.3.1. Interleukin-6 measures

At 2, 4, and 7 months of age, whole blood was collected from undisturbed animals, diluted 1:1 with IMD buffer, and separately stimulated with phytohemagglutinin (PHA; 5 μg/mL) for 48 h, LPS (10 ng/mL) for 24 h, or with saline. IL-6 levels in the supernatant were then quantified by ELISA. Blood samples were also cultured immediately after the HIP challenge test at 8–9 months of age. Baseline blood levels of IL-6 *in vivo* were determined at 1 year of age and after administration of 4 ng/kg LPS i.v. (at 1.5 years of age) to evaluate if the monkeys showed signs of a possible endotoxin tolerance or sensitization [32]. Plasma IL-6 levels were determined at 1 and 3 h after this injection of LPS.

2.3.2. Cortisol measures

Adrenal hormone levels were determined under basal and challenge conditions using a week-long protocol [21]. Briefly, cortisol was assessed at baseline, after transfer to a novel cage, 2 days later following acclimation, and on the day after overnight dexamethasone treatment.

2.4. Neuroimaging

At approximately 1 year of age, T1 and T2-weighted neural images were acquired using a GE Signa 3-T scanner (General Electric Systems, Milwaukee, WI). One animal was re-scanned at 1.5 years of age due to a positioning error of its head in the stereotax platform, which lead to inadequate normalization to the brain template; the new scan acquisition and the variation in age at scan did not bias group differences. For the neuroimaging scan acquisition, the animals were initially anesthetized by administration of ketamine hydrochloride (10 mg/kg, i.m.) followed by medetomidine (50 μg/kg, i.m.) and placed into an 18 cm quadrature extremity coil (IGC Medical Advances, Milwaukee, WI). Details of the scan acquisition and data analysis have been described previously [21,33]. For the T1-weighted scan, a high resolution axial Inversion Recovery-prepped 3D-SPGR sequence was used: inversion time = 600 ms; TR = 8.6 ms; TE = 2.0 ms; FOV = 160 mm; flip angle = 10°; matrix = 256 × 256 × 124; slice thickness = 1.5 mm; slice gap = −.5 mm; bandwidth = 15.63; voxel resolution of .234 mm × .234 mm × .498 mm. The T2-weighted scan had the following parameters: TR = 12,000 ms; TE = 92.8 ms; FOV = 160 mm; flip angle = 90°; matrix = 512 × 512; slice thickness = 1.5 mm; slice gap = 0 mm; bandwidth = 31.25; voxel resolution of .27 mm × .27 mm × 1.5 mm. T1 and T2 weighted scans were aligned using a 3-point localizer during the scanning session. For image preprocessing, an automated method was first used to skull-strip a given brain, followed by affine coregistration and then non-linear 12 parameter normalization to a customized juvenile rhesus monkey template [34]. T1 and T2 probability maps were jointly used for segmentation of GM, WM, and cerebrospinal fluid (CSF). Fig. 1 depicts the segmentation of tissue classes for regional parcellation and the derivation of cortical thickness [34,35]. Volume was derived for each parcellated region of interest for GM and WM segments.

2.5. Statistical analyses

Analyses were conducted using SPSS 15.0 (Chicago, IL). Effects of Prenatal Condition (LPS vs. Control) on behavior or physiology across Age or Trials were analyzed with either mixed repeated measures analysis of variance or covariance (ANOVA, ANCOVA) or by an omnibus *F*-test. Multiple scores in behavioral tests were collapsed together to improve homoscedasticity of variance and normality. Square root or base log 10 transformations corrected variables that deviated from normality, homoscedasticity, or sphericity. Two-tailed *t*-tests were used in post hoc analyses of individual variables. Omnibus repeated measures tests were used to guard against experiment-wise error for neural region of interest analyses. Intracranial volume (ICV)-corrected values were also examined. Alpha level was set at .05.

3. Results

3.1. Maternal response to LPS and neonatal health

Compared to pre-injection blood levels, administration of LPS resulted in a significant increase in IL-6 levels in the gravid females [$F(1,8) = 25.10$, $p = .001$]. An acute increase in neutrophils [$F(1,8) = 16.64$, $p < .01$] and decrease in lymphocytes [$F(1,8) = 40.99$, $p < .001$] were evident in all LPS-injected animals. Post hoc tests confirmed these changes for Day 1 and 2 (see Supplement Table 1).

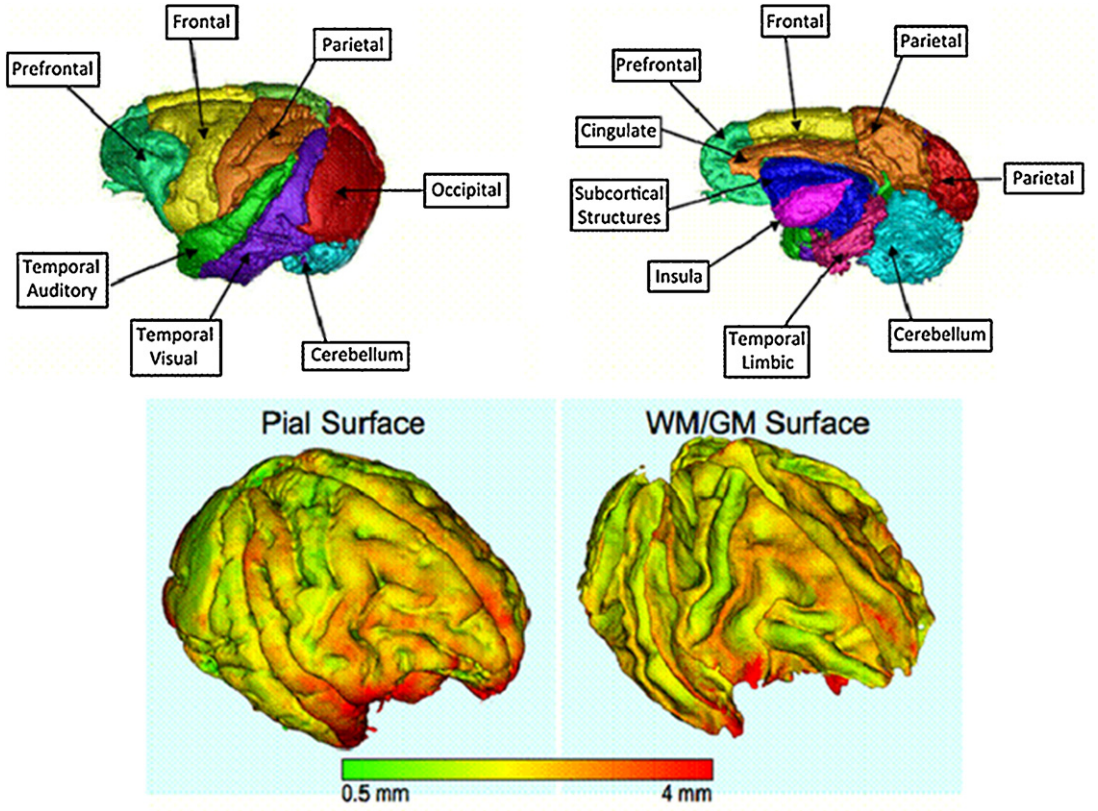

Fig. 1. Representative segmentation and parcellation of the brain into regional GM and WM volumes, respectively (top row). Cortical thickness was also determined in these hemispheric regions by examining the distance between the pial and WM surfaces (bottom row) [32].

Physiological values in control animals remained similar to baseline across the 2 days. All infants were born normally without delivery complications. Prenatal LPS treatment did not affect gestation length, neonatal weight, and subsequent growth patterns. No significant differences were noted in maternal care or rate of infant maturation.

3.2. Neuroimaging

When scanned at 1 year of age, the ICV of monkeys from the LPS-treated pregnancies was marginally 5.9% larger than for the control monkeys (Tables 1 and 2). More dramatically, LPS monkeys had a significant 8.8% increase in mean global WM volume.

Table 1
White matter volumes, both globally and in cortical regions and select neural structures, for control ($n = 9$) and LPS ($n = 9$) progeny.

Neural area	Hemisphere	Control mean (voxels)	LPS mean (voxels)	p-value ICV uncorrected	p-value ICV corrected
Intracranial volume		88,512 ± 1194	93,726 ± 2625	≤.09	N/A
Global WM		20,068 ± 285	21,839 ± 738	≤.05	≤.06
Cortical regions					
Prefrontal	Left	712 ± 18	829 ± 30	≤.01	≤.001
	Right	708 ± 18	812 ± 29	≤.01	≤.01
Frontal	Left	1260 ± 21	1430 ± 52	≤.01	≤.01
	Right	1263 ± 21	1419 ± 55	≤.05	≤.05
Cingulate	Left	139 ± 3	160 ± 6	≤.01	≤.07
	Right	129 ± 4	151 ± 6	≤.01	≤.09
Temporal auditory	Left	382 ± 9	447 ± 18	≤.01	≤.01
	Right	388 ± 8	448 ± 19	≤.01	≤.05
Temporal visual	Left	1336 ± 23	1414 ± 39		
	Right	1344 ± 22	1415 ± 41		
Medial temporal	Left	129 ± 6	161 ± 9	≤.01	≤.05
	Right	161 ± 6	195 ± 11	≤.05	≤.05
Parietal	Left	1574 ± 31	1730 ± 67	≤.05	
	Right	1605 ± 28	1761 ± 61	≤.05	≤.09
Occipital	Left	1315 ± 39	1362 ± 33		
	Right	1397 ± 44	1452 ± 30		
Subcortical regions					
Corpus callosum		577 ± 23	649 ± 30	≤.09	
Cerebellum	Left	1071 ± 22	1094 ± 37		
	Right	1082 ± 23	1115 ± 36		
Brainstem		1360 ± 35	1453 ± 59		

Mean ± SEM is reported for raw WM volume estimates of monkeys from control and LPS pregnancies. The absolute value of each region of interest was divided by subject's ICV to assess differential effects on each region relative to total brain effects. Blank spaces signify non-significant results.

Table 2
Gray matter volumes, both globally and in cortical regions and select subcortical structures, for control ($n = 9$) and LPS ($n = 9$) progeny at 1 year of age.

Neural area	Control mean (voxels)	LPS mean (voxels)	p-value ICV uncorrected	p-value ICV corrected
Intracranial volume	88,512 ± 1194	93,727 ± 2625	≤.09	N/A
Global GM	50,648 ± 792	53,432 ± 895		
Cortical regions				
Prefrontal	5055 ± 127	5501 ± 242		
Frontal	5436 ± 129	5977 ± 191	≤.05	≤.05
Cingulate	1628 ± 23	1696 ± 63		
Temporal auditory	3745 ± 78	3911 ± 128		
Temporal visual	5605 ± 99	5863 ± 192		
Medial temporal	2217 ± 43	2286 ± 57		
Parietal	5812 ± 133	6385 ± 234	≤.05	≤.07
Occipital	8066 ± 156	8211 ± 252		≤.06
Sub-cortical regions				
Caudate	606 ± 12	624 ± 16		
Putamen	860 ± 11	921 ± 24	≤.05	
Hippocampus	400 ± 5	429 ± 11	≤.05	
Amygdala	381 ± 6	402 ± 11		
Cerebellum	4212 ± 98	4536 ± 157		
Brainstem	226 ± 8	230 ± 9		

Mean ± SEM is reported for raw GM volume estimates of monkeys from control and LPS pregnancies. The absolute value of each region of interest was divided by subject's ICV to assess differential effects on GM of each region relative to global GM. The small size of the insula precluded accurate estimation and analysis. Blank spaces signify non-significant results.

Global GM, total CSF, and ventricular size did not differ statistically from controls. Variations in age at scan did not account for these results. Omnibus tests were conducted to guard against type 1 error and justify volumetric analyses for regions of interest (see Supplement Text 1). Nearly all WM regions were significantly larger in LPS-exposed monkeys, whereas selective GM changes were seen in parietal and frontal areas (Tables 1 and 2; Fig. 2), as well as in hippocampus and putamen. The findings for cortical thickness supported the GM results (Supplement Table 4), with marginally thicker GM in the right parietal and frontal lobes, but thinner GM in medial temporal lobe. To assess the relative magnitude of the regional changes, the volumes and cortical thickness were divided by the monkey's ICV or its cube root, respectively. The statistical significance of volumetric and cortical thickness results remained largely unchanged.

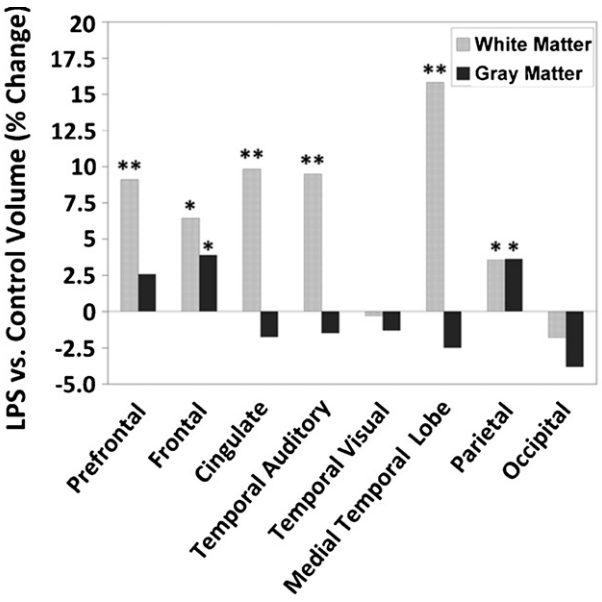

Fig. 2. The percentage change in gray matter and white matter cortical volumes induced by prenatal LPS treatment ($n = 9$) relative to controls ($n = 9$). $^{*}p < .05$, $^{**}p < .01$.

3.3. IL-6 and cortisol levels

As detailed in Supplement Figs. 1 and 2, there was a relatively mild impact on pituitary-adrenal activity and a complex bidirectional effect on IL-6 responses in LPS-exposed offspring over time. When monkeys from the LPS-treated pregnancies were moved to a new cage, their cortisol levels were higher after 2 days relative to baseline, as compared to hormonal adaptation of control animals. Following overnight dexamethasone treatment, the morning cortisol levels of the LPS-exposed monkeys were initially more suppressed, but by afternoon the cortisol levels were elevated when compared to controls. While with their mothers at 2 and 4 months of age, infants from the LPS condition initially appeared to show more cellular reactivity when their blood was stimulated *in vitro* with PHA. However, 1 month after being weaned from the mother, their cellular response to PHA as reflected by IL-6 in the supernatant was significantly lower than those of control offspring.

3.4. Behavior

3.4.1. IBAS at 2 weeks of age

Some behavioral differences were already evident at 2 weeks of age, when infants from LPS-treated pregnancies received higher Emotionality ratings during the IBAS test [$t(15) = 3.17$, $p < .01$]. One LPS offspring was excluded from this analysis because it appeared to become weak and fatigued by the testing. This exclusion did not influence the significance of results. A repeated measures omnibus on all variables constituting this Emotionality factor [$F(1,15) = 6.88$, $p < .05$], followed by t-tests, showed that LPS offspring were also significantly hyperresponsive for the constituent test items and related measures such as vocalizations (see Supplement Table 5). No other differences in behavioral maturation or activity were evident at this age.

3.4.2. Social interactions during first 7 months of age

No overt effect of the prenatal LPS treatment was seen on infants' social and exploratory behavior while observed undisturbed with the mother or after weaning into small peer groups.

3.4.3. HIP behavior and post-HIP IL-6 response at 8–9 months of age

As detailed in Fig. 3, 8–9–month old LPS offspring exhibited a pattern of marked behavioral reticence in contrast to their earlier

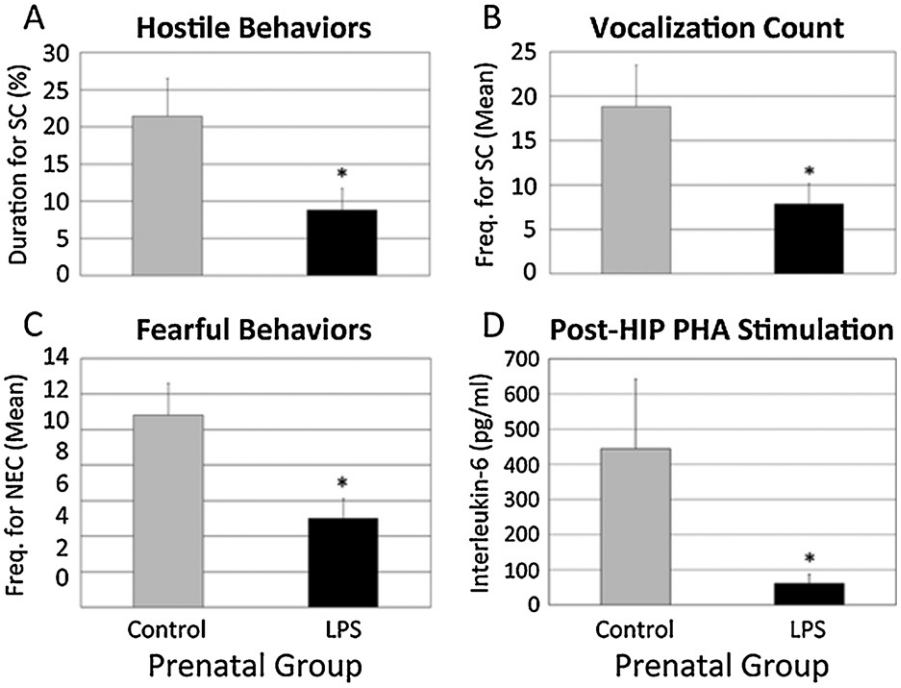

Fig. 3. Frequency and percentage duration of HIP behavior during the stare challenge (SC) and no eye contact (NEC), as well as the post-HIP *in vitro* assessment of IL-6 production. Monkeys from the control ($n = 9$) and LPS ($n = 9$) pregnancies were assessed. See Supplementary Table 3 for the list of behaviors recorded. Data are depicted as mean ± SEM. *Student's *t*-test, $p < .05$.

reactions during the IBAS. While this test typically evokes a range of anxious and hostility behaviors that are listed in Supplement Table 3, the LPS progeny showed these behaviors less frequently than controls (e.g., vocalizations), both during the NEC and SC phases despite more exploratory activity during the baseline phase. Specifically, at baseline, LPS animals initially engaged in more tactile and oral exploration of their environment: 10% of the time compared to 4.3% for controls [$t(16) = 2.81$, $p < .05$]. Both groups spent the remainder of this period predominantly moving and did not differ significantly. Following entrance of the experimenter into the room, LPS offspring became more behaviorally reticent during the SC and NEC phases. During the SC phase, a Stress Behavior × Prenatal Condition interaction [$F(1,16) = 3.09$, $p < .05$], followed by post hoc testing, showed that the LPS animals spent less time or performed fewer bouts of freezing [$t(16) = 2.23$, $p < .05$], self-contact [$t(16) = 3.05$, $p < .01$], and experimenter-oriented fixation [$t(16) = 2.19$, $p < .05$]. They also engaged in less hostile behavior toward the observer [$t(16) = 2.41$, $p < .05$], represented as a mean percentage of time spent during a 300 s trial (Fig. 3A). As compared to controls, the mean frequency of vocalizations was less during the SC phase [$t(16) = 2.41$, $p < .05$] (Fig. 3B). Monkeys from LPS-treated pregnancies also appeared less responsive during the NEC phase, during which they froze less often [$t(16) = 2.75$, $p < .05$] and engaged in less visual fixation toward the experimenter [$t(16) = 2.75$, $p < .05$]. The mean frequency of fearful non-vocalization behaviors is depicted in Fig. 3C. Immediately after the HIP, blood was drawn and stimulated with phytohemagglutinin (PHA) to examine IL-6 levels. When blood was collected immediately after the test and stimulated *in vitro* with PHA, there was a significantly greater stress-related suppression of IL-6 release in the blood taken from LPS-exposed monkeys [$t(13) = 2.16$, $p < .05$] (Fig. 3D).

3.4.4. Acoustical startle and PPI at 10–12 months of age

When PPI tests were conducted at 10–12 months of age, monkeys from the LPS-treated pregnancies were found to freeze and startle less than controls across the four initial 115 dB pulses [$F(1,13) = 11.56$, $p < .01$]. Mean pulse-induced startles were reduced by 55% across all subsequent pulse trials [$F(8,104) = 2.35$, $p < .05$] (Fig. 4A). For %PPI at 115 dB, a repeated measures omnibus indicated that juvenile monkeys from LPS pregnancies had a dysregulated

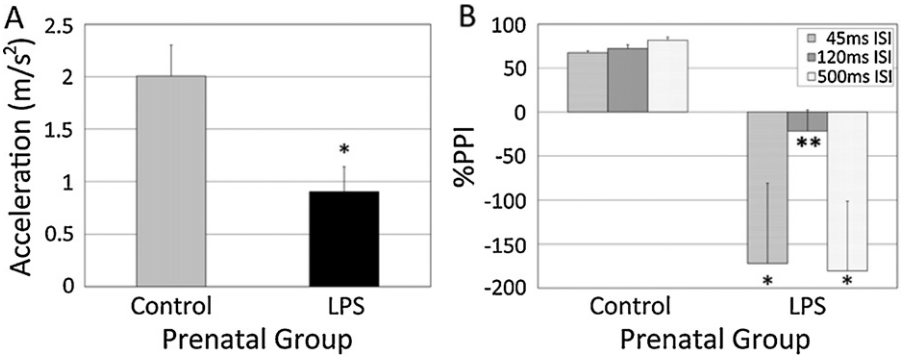

Fig. 4. Behavioral responses during the prepulse inhibition (PPI) test among LPS treatment ($n = 6$) and control ($n = 9$) monkeys. (A) Mean startle reaction to a pulse sound probe during the PPI. (B) Change in startle response following an acoustic prepulse (percentage PPI) calculated from the prepulse trials combined for the 105 and 115 dB pulses. Data are depicted as mean ± SEM. *$p < .05$; **$p < .01$ (Student's *t*-test).

response following the prepulse across the 3 inter-stimulus intervals (ISI) [$F(1,13) = 9.99$, $p < .05$]. Specifically, for these monkeys, the prepulse generally did not suppress but rather augmented the startle—a facilitative event noted by others after some prenatal challenge paradigms [18]. Post hoc analyses indicated that this change in %PPI was evident at the ISI of 45 ms [$t(13) = 2.46$, $p < .05$], 120 ms [$t(13) = 3.45$, $p < .01$], and 500 ms [$t(13) = 2.73$, $p < .05$]. Similarly, at 105 dB, LPS animals had a potentiated startle [$F(1,13) = 7.01$, $p < .05$]. This effect occurred at 45 ms [$t(13) = 2.45$, $p < .05$] and 120 ms [$t(13) = 2.94$, $p < .05$]. The startle data for 105 and 115 dB probes were also analyzed altogether. The joint analysis indicated that the LPS-induced change in %PPI was significant for all 3 ISIs at 45 ms [$t(13) = 2.72$, $p < .05$], 120 ms [$t(13) = 3.02$, $p < .01$], and 500 ms [$t(13) = 2.60$, $p < .05$]) (Fig. 4B). Three LPS monkeys manifested high reactivity to prepulse-pulse trials in the 45 ms and 500 ms ISI trials, producing wide variance.

4. Discussion

Our study has generated novel findings on the neural effects of a mild endotoxemia paradigm in pregnant monkeys using nanogram amounts of LPS. Specifically, this 2-day treatment expanded WM volume in many regions and selectively enlarged GM in the infants from LPS-treated pregnancies. This neurodevelopmental profile bears some similarity to the early brain overgrowth described in many individuals with autism [22,23]. In contrast, endotoxin and viral infection models in rodents typically result in reduced neural growth or no effect depending on the gestational timing and species [2]. Even though the brain effects in monkeys were opposite from the typically reported direction, the behavioral profile of these offspring from the LPS-treated pregnancies appeared comparable to several rodent models of prenatal infection and stress [36,37].

A similar experiment by our laboratory involving influenza virus infection during this period of gestation induced the more commonly observed reduction in the neural parenchyma [21]. Thus, it does not seem that differences in gestational timing and fetal brain maturation between monkeys and rats account for the differential outcome [38–41], but rather the degree of maternal inflammation induced by the various paradigms. Most mouse and rat models use concentrations of endotoxin in the microgram to milligram range, which can cause widespread physiological activation, including a marked retardation of fetal growth and some fetal death [42]. By contrast, the IL-6 levels post-LPS were only modestly upregulated in gravid monkeys over 2 days. Nevertheless, the elevated IL-6 likely crossed into the amniotic fluid and fetal compartment and was sufficient to induce the observed neurodevelopmental changes [12,43]. Indeed, IL-6 knockout mice are less likely to show neurobehavioral effects after prenatal inflammatory challenges [12]. It is not clear if the LPS-induced brain differences observed in our monkeys when they were approximately 1 year of age reflect an early overgrowth or an accelerated maturation that might persist into adulthood as seen in some rodent and monkey models [9,22,44,45]. The overall brain size and WM volumes of the LPS-exposed monkeys were roughly 6 months ahead of comparably aged infants from normal pregnancies [33].

The most striking finding in the offspring from the prenatal LPS condition was the significant 8.8% increase in global WM volume and a trend toward whole brain enlargement. As noted above, these results differed from the commonly reported decrements in brain size and neural development in rodents and sheep following *Escherichia coli*, and other teratogenic and inflammatory stimulation [19,46–48]. Regional WM was increased throughout the hemispheres, especially in the more rostral and temporal areas. The effects on absolute GM and WM volume were not evident in the occipital lobe, however, which matures early in the fetal monkey and thus may have been spared [49]. This enlargement may be due to interference with the dendritic and synaptic pruning that normally occurs during gestation [50,51]. Alternatively, prenatal LPS may have accelerated the rate of myelination postnatally. Pro-oligodendrocyte precursors can be stimulated by certain mitogens and inflammatory stimuli [52,53] and could have resulted in enhanced myelin development.

Circumscribed increases in GM volume and, to a lesser extent, in cortical thickness were also seen in the parietal and frontal lobes, to approximately 4% more than controls. Alcohol or stress exposures typically reduce volume in these regions [54,55]. Our previous study on influenza virus infections [21] also found a decreased volume of approximately 6% and 10% for frontal and parietal lobes, respectively. Increased subcortical volumes were also seen in hippocampus and putamen in offspring from the prenatal LPS condition. It has been shown that LPS exposure to mice on gestational day 17 can increase cell density within the CA fields [56], and enlargement of the hippocampus is noted in some children with autism [57,58] – although others have noted no changes [59] or decreased GM relative to total brain volume [60]. In contrast to the general enlargement, the decreased cortical thickness in the medial temporal lobe of the LPS-exposed monkeys has been observed in human paradigms [55]. Damage to medial temporal lobe including entorhinal and perirhinal cortices can disrupt sensorimotor gating [61] and also sensitize rhesus monkeys to aversive stimuli [62]. LPS-induced disruption in the neurocircuitry or GM of this area could underlie the changes in behavioral reactivity observed in the LPS-exposed offspring under arousing conditions.

Changes in the monkeys' temperament were evident throughout development, which corresponded to our periodic finding of physiological differences in IL-6 levels. LPS infants showed heightened responsiveness (e.g., more vocalizations) during the IBAS testing at 2 weeks of age, whereas they later became behaviorally reticent (e.g., fewer vocalizations) during the HIP at 8–9 months. Following weaning from the mother, and also immediately after the HIP test, there was a greater stress-induced inhibition of PHA-stimulated IL-6 production *in vitro*. They also startled less to PPI test pulses and failed to manifest the typical adaptation after prepulse sounds. These behavioral effects are similar to deficits in rodents prenatally treated with proinflammatory cytokines or endotoxin [12,18]. These effects are also reminiscent of the poorer behavioral modulation seen in inhibited children at risk for affective psychopathology [63], although this animal model did not replicate several of the key features of autism, such as social and communication deficits. The impact of our prenatal treatment on HPA activity was also less than described for most rodent endotoxemia models [64,65], but the LPS-exposed animals did take longer to adapt to transfer into a novel cage and had a differential response to negative glucocorticoid feedback after overnight Dexamethasone treatment.

5. Conclusion

In summary, a 2-day endotoxin provocation during pregnancy had a striking impact on many brain regions, increasing GM and WM volumes, and altering cortical thickness. Notwithstanding these marked changes in the brain phenotype, the LPS-exposed monkeys were healthy and appeared behaviorally and physiologically normal until examined under arousing and challenging conditions. This new primate model may afford an opportunity to examine processes that mediate the neural overgrowth seen in some neurodevelopment disorders such as autism, where problems with attention and reactivity are sometimes associated with regional increases in either cortical GM or WM [66–68], as well as in subcortical structures including hippocampus and putamen [22,58,66].

Disclosure/conflict of Interest

The authors have no conflicts of interest regarding this manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbr.2010.12.023.

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